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Transcapillary Loss, Equilibrium Time, Half-Return Time of Thiocyanate and Heavy Water in the Forearm.* (22424)

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We previously described a method for estimating the transcapillary exchange of permeable substances in the human forearm(1). The method later was extended to the pulmonary capillaries of man(2). No attempt was made in these experiments to estimate the length of time the permeable substances remain in the tissues or their rate of return to the circulation. More recently we have described a method for determining the late washout slopes of injected tracer materials in the human forearm(3). The method takes advantage of the fact that the forearm circulation is small in comparison to the general circulation. Thus, brachial arterial injection of tracer materials in dosages sufficient to produce significant concentrations in the effluent ipsilateral veins produces negligible concentrations when diluted in the general circulation. In this way contamination and consequent distortion of the late washout slopes due to recirculation of significant amounts of labelled material can be avoided.

The present report is concerned with the transcapillary loss and later return of the extracellular electrolyte, thiocyanate, and of heavy water. We believe that these studies provide a more complete picture of the circulation of extravascular substances under physiological circumstances than has been available heretofore.

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Method. Preparation of labelled materials and manner of injection and sampling have been described in other communications(4,1, 3). The method of analysis of thiocyanate and deuterium oxide also has been described previously(1). The subjects were young or early middle-aged males who were on the wards of the Veterans Administration and Georgetown University Hospitals. They all were ambulatory and afebrile at the time of testing and were not suffering from circulatory disease which might interfere with normal transcapillary exchanges in the forearm. Twenty-two tests were carried out using T-1824 and thiocyanate. In 8 subjects D_2O was determined. In 13 tests forearm and hand capillary beds were included. In one of these subjects (M.W., Table I) simultaneous sampling was carried out from cephalic and median veins. In 8 subjects tracer materials were limited to the forearm by inflating a cuff about the wrist to pressures of 100 mm Hg above systolic pressure. In the remaining 2 cases, permeability characteristics of the hand alone were studied, injection being made into a radial artery with subsequent sampling from a vein near the wrist. The site of transcapillary exchange (forearm or hand or both) did not appear to make any significant difference in the results obtained.

Definition of Terms. Methods for determining rate of net return have not been described previously and, therefore, requires some explanation. The method of determining transcapillary percentage loss has been

described previously(1) so that its principles only will be briefly outlined here. Mere injection of a permeable substance with later sampling is not adequate because it is impossible to determine the extent of its dilution by the blood. The present technic combines the permeable tracer materials with an impermeable substance (T-1824). A portion of this mixture is injected into the brachial artery and another portion saved for analysis of the relative concentrations of the various substances in the injectate. 1. *Expected concentration* of each permeable tracer is the concentration of the substance in each sample that would be expected if there were no transcapillary gain or loss as the material passed through forearm circulation. The permeable labelled substance is mixed completely with the impermeable tracer prior to injection. It is assumed that the two travel together and are equally diluted by the circulating blood subsequent to injection into the brachial artery. It does not matter if the pattern of blood flow distribution is uneven in the forearm so long as the impermeable and permeable tracer are distributed to each vascular bed in the same proportionate concentration as was present in the injectate. The plasma samples collected at intervals of 2 to 4 seconds from an effluent vein are analyzed for their concentration of the impermeable tracer (T-1824). Concentrations of both permeable and impermeable substances also are determined in an aliquot of the injected mixture. The expected concentrations of the permeable substance in each sample are calculated as follows: $C^t_x = \frac{C_x}{C_e} \times C^e_m$ where C^t_x is the expected concentration of the permeable substance in each sample, C_x the concentration of this substance in the injectate, C_e the injectate concentration of the impermeable tracer and C^e_m the respective sample concentration of the impermeable material. Suitable correction must be made for red cell penetration as has been described in a previous report(1). The venous samples also are analyzed for their *actual* concentrations of each permeable tracer. The values of expected and actual concentrations then are plotted on semi-log paper as indicated in Fig. 1.

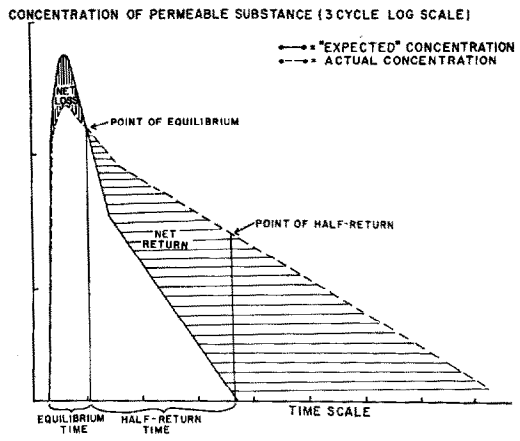

FIG. 1. Graph of "expected" and actual concentrations of a hypothetical permeable labelled substance illustrating the terminology employed. See "Definition of Terms" section in the text for details.

2. *Equilibrium Time.* It will be seen from Fig. 1 that values of actual concentration at first lie below and later cross over to remain above the curve of expected concentrations. The difference between the expected and actual concentrations to the left or prior to the point of crossing represents net losses from blood to the extravascular tissues whereas the difference between the actual and expected concentrations to the right of the crossing indicates net return from the tissues to the blood. The point of crossing therefore represents an equilibrium and the time from appearance to the point of crossing is called the *Equilibrium Time* of the respective permeable tracer. It also represents the total period of net transcapillary loss. 3. *Half-Return Time.* The area enclosed by the respective curves of expected and actual concentrations between the appearance and the point of equilibrium is proportional to the total net loss in the vascular bed drained by the effluent vein used for sampling. The latter is calculated by integrating the net losses per unit time from appearance to equilibrium time (expected minus actual concentrations per unit time). The net returns per unit time (actual minus expected concentrations beginning at the equilibrium point) then are added together until the sum equals half the total net loss. Since

net loss begins at the equilibrium point the time of half return minus the time of the equilibrium point is designated as the *half-return time*. It represents the duration of time in seconds from the beginning of net return to completion of half-return. Since there is no significant recirculation of the tracer materials it is possible to estimate the return to the blood stream of the substances which have permeated into the tissues. Following the passage of the main bolus of injectate through the capillaries of the forearm the subsequent blood entering this vascular bed contains negligible quantities of the permeable tracer materials. This produces a favorable gradient across the capillary wall for determining the rate of return of the permeable tracer substances to the circulation.

Results. Early per cent loss of thiocyanate and deuterium oxide. During the early portion of the transit curves of the labelled materials up to and including the peak values the concentrations in the blood are continuously rising and exceed those in the tissues. Thus, during this period there is a gradient between blood and tissues favoring outward migration of the permeable substances and opposing their inward return. The percentage loss of the SCN and D_2O during this early period, therefore, probably represents a fair approximation of the permeability of the capillary wall to these substances. The percentage losses of SCN and D_2O were determined by dividing the difference between the expected and actual concentrations by the expected concentrations(1). The results listed in Table I represent the percentage losses at the peak. For thiocyanate the mean transcapillary loss was 49 $\pm$ 19%. For deuterium oxide the mean loss was 90 $\pm$ 4.1%. These values are not materially different from those previously reported in a smaller series (1).

Equilibrium time of thiocyanate. The upslope and early downslope of the SCN concentration curve paralleled the T-1824 curve. However, after the early downslope SCN deviated to produce a washout slope with a smaller gradient than that of T-1824. When the expected concentrations of thiocyanate

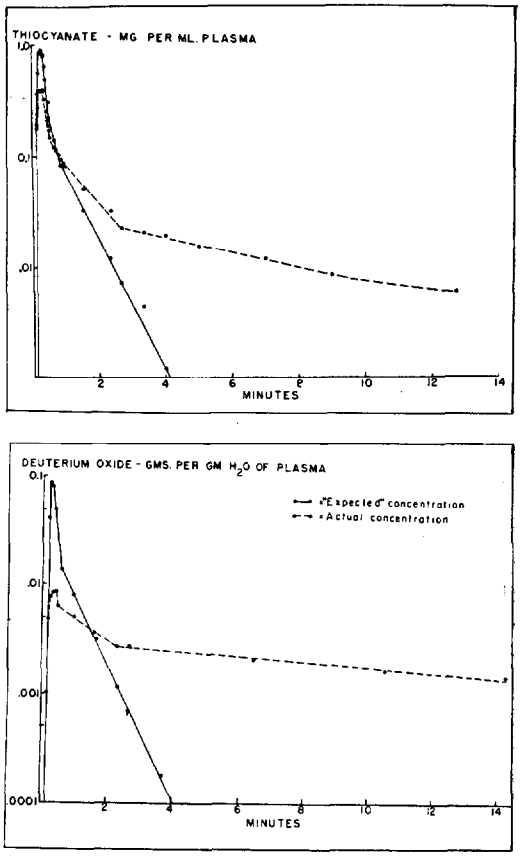

FIG. 2. (Upper graph.) The curves of expected (solid line) and actual (broken line) concentrations are plotted against time. Simultaneously determined curves for deuterium oxide are plotted in lower graph. 19th line of Table I.

were plotted against the actual concentrations the curves crossed at a point in their downslopes as shown in Fig. 2a. This crossing represents the time at which the net loss of thiocyanate equals the net gain (see under "definition of terms," above). The equilibrium time or period of net loss for SCN in the 22 subjects averaged 111 $\pm$ 66 seconds (Table I). The equilibrium time for SCN varied directly with the mean circulation time (T) of T-1824. When the equilibrium times of SCN were plotted against the respective mean circulation times of T-1824 the points were grouped along the line shown in Fig. 3a. The formula for this line indicated that the equilibrium time of SCN was approximately equal to 3 times the mean circulation time of

TABLE I. Per Cent Losses, Equilibrium Times and Half-Return Times for Thiocyanate and Deuterium Oxide in 22 Subjects.

		Thiocyanate				Deuterium oxide				
Age	$T_{T\text{-}1824}$,§ sec.	% loss at peak	E.T.,* sec.	0.5 RT, sec.	0.5 RT† /E.T.	% loss at peak	E.T., sec.	0.5 RT, sec.	0.5 RT /E.T.	Comment
27	72	68	60	78	1.3					
28	35	61	25	50	2.0					
30	79	70	155	1596	10.3					
30	27					87	24	30	1.25	
33	29	53	27	43	1.6	97	43	69	1.6	
43	125	49	215	151	.7	83	270	567	2.1	
31	51	56	21	103	4.9					
31	61	55	98	265	6.8					
28	63	80	145	1218	8.4					
27	38	44	24	38	1.6					
34	92	55	193	283	1.5	90	203	587	2.9	
30	56	49	39	113	2.9					Cephalic vein drainage
"	65	46	105	147	1.4					Median vein drainage
31	41	50	125	1375	11.0					Hand circulation only
40	67	40	160	224	1.4					*Idem*
36	35	56	33	92	2.8					Forearm only
45	114	67	220	418	1.9					*Idem*
33	102	61	160	224	1.4	87	185	518	2.8	"
32	32	56	36	209	5.8	91	85	196	2.3	"
38	70	48	146	‡						"
30	90	42	185	389	2.1					"
28	39	46	57	97	1.7	91	82	158	1.9	"
24	61	54	90	216	2.4	91	145	320	2.2	"
Mean	61	49	111	370	3.0	90	130	306	$2 \pm .6$	
S.D.	± 29	± 19	± 66	± 199	± 3.0	± 4	± 86	± 256		

* E.T. = Equilibrium time. † 0.5 RT = Half-return time. ‡ Too long to calculate.
§ $T_{T\text{-}1842}$ = Mean circulation time of T-1824.

the plasma (T-1824) minus 75 seconds. All of the values fell within the range of $3T_{T\text{-}1824}$–(0 to 150 seconds).

Half-return time of thiocyanate. The half-return time of SCN was determined in each case according to the method outlined under "definition of terms," above. The mean half-return time for SCN in the 22 subjects was 370 ± 199 seconds (Table I). The period from appearance to half-return time averaged approximately 7 to 8 minutes. Since the equilibrium time represents the period during which net loss occurred it was of interest to compare it to the half-return time. In 20 cases the half-return time for thiocyanate ranged between 0.7 to 10.3 times the equilibrium or loss time with a mean of 3 ± 3.0 (Table I). Thus, even with a favorable concentration gradient the half return of this extracellular substance to the circulation averaged 3 times longer than the period of transcapillary loss.

Equilibrium and half-return times of heavy water. The great facility with which heavy water passes through semi-permeable membranes was shown not only by the early high percentage losses of this substance but also by the curves of expected and actual concentrations. In Fig. 2b and in most of the other cases as well the peak of the curve of actual concentrations occurred approximately 10 seconds later than the peak of the expected. This was interpreted as indicating a massive buildup of D_2O outside the capillaries at the time of peak passage of the bolus of injectate followed by rapid re-entry of a portion of this extravascular D_2O. This delayed peak of actual concentrations never was observed with SCN in the forearm circulation suggesting more hindrance to back diffusion of SCN than of D_2O. The larger space into which D_2O permeates was indicated by the some-

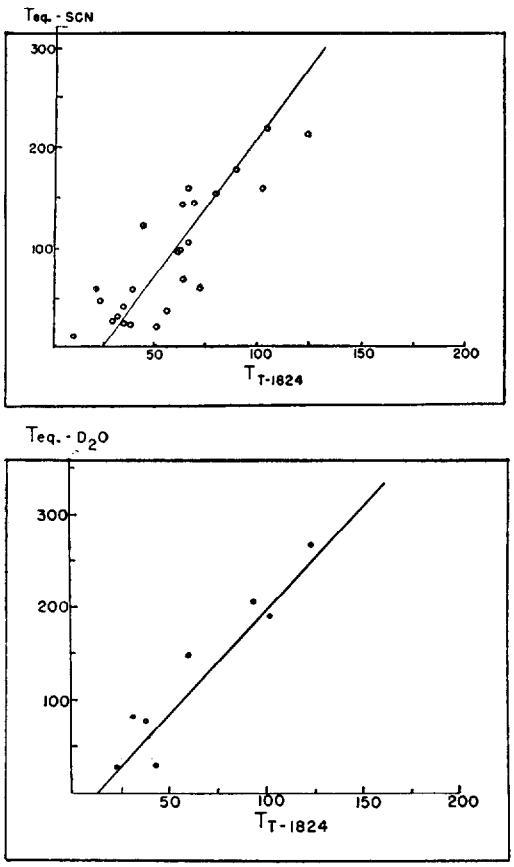

FIG. 3. (Upper graph.) The equilibrium times for thiocyanate are plotted against the respective mean circulation times of T-1824. The equation of the middle line is $T_{eq_{SCN}} = 3T_{T\text{-}1824}$ minus 75 seconds. (Lower graph.) Similar values for D_2O are plotted. The equation of this line is $T_{eq_{D_2O}} = 2.5$ $T_{T\text{-}1824}$ minus 31 seconds.

what longer equilibrium and half-return times for D_2O than for SCN. The equilibrium times of D_2O averaged 130 ± 86 seconds (Table I). These were related to the mean circulation times of the plasma as is indicated in Fig. 3b. Equilibrium time D_2O averaged 2.5 $T_{T\text{-}1824}$–31 seconds (range –7.5 to 70 seconds). In 7 cases SCN and D_2O were injected simultaneously. In these the equilibrium times D_2O averaged only 35 per cent more than the equilibrium times SCN. This again indicates the remarkable diffusion characteristics of heavy water through semi-permeable membranes since D_2O passes through cellular membranes and its extravascular space is approximately three times the SCN extravascular space.

In the 7 cases in which SCN and D_2O were injected simultaneously the half-return time of thiocyanate averaged 175 seconds and the half-return time of heavy water averaged 345 seconds. Thus, the mean half-return time D_2O was approximately twice as great as that of SCN.

The mean half-return time of D_2O in the 8 cases studied was 306 ± 256 seconds (Table I). This represented 2.1 ± 0.6 times the equilibrium time. Although these values are somewhat lower than the corresponding means for SCN, inspection of Table I will show that the latter were heavily weighted by a few cases with greatly prolonged half-return times. In all of the 8 cases the time from appearance to half-return of D_2O was less than 10 minutes.

Discussion. The validity of the method for determining percentage loss of the permeable tracers has been discussed previously (1). The method now has been extended to provide information on the rate of net exchange in both directions across the capillary membrane.

The deviations of the actual from the expected concentrations can only be explained by loss or gain to the circulating blood. Part of this loss could be into the walls of the blood vessels themselves which still would represent penetration into or through an endothelial membrane. It seems probable such losses are negligible compared to the transcapillary exchange.

The method does not provide information on the total exchanges in the forearm since the distribution of the labelled materials may not be evenly dispersed throughout all of the forearm vessels(5). However, since impermeable and permeable tracer substances are completely mixed prior to injection it is valid to assume that they will be delivered to the capillary beds of the area being sampled in the same relative concentrations as were present in the injectate.

The present state of knowledge regarding capillary permeability has been reviewed recently by Pappenheimer(6). Comparison of the present results with those reported previ-

ously in the literature is difficult because of differences in approach and technic. Much of the prior work has attempted to express the permeability characteristics of biological systems using the terminology employed in the physical sciences. The convention of using permeability constants representing the number of moles of a substance which cross unit cross-sectional area of the membrane in unit time under unit concentration difference may be useful in model systems where these variables are known. However, in biological systems they are not known and the attempt to estimate them introduces questionable assumptions and difficult complexities.

In regard to the organ systems of man we essentially are desirous of knowing (1) the magnitude of transcapillary loss of various substances and the factors which influence them, (2) the extravascular distribution of these substances, and (3) their "half life" in the tissues or their rate of return to the blood. Progress in this field especially in relation to the study of disease states depends upon the development of relatively direct and simple methods. Such methods need not mimic the approach used by the purely physical sciences where the experimental circumstances are completely different.

The net transcapillary loss of heavy water was approximately 90% and its return to the circulation was relatively rapid. The high rate of loss is consistent with previous observations from this laboratory and also with those of Chinard(7). Such high percentage losses and rapid returns are consistent with a process of diffusion rather than "filtration in bulk"(8). The half-return time of water was short when one considers that it also penetrates into cells. The present data suggest that more than half of the tissue water exchanges across the capillaries several times in an hour. It seems likely that this great movement which must include intracellular water serves as the medium through which much of the cellular metabolism is accomplished.

Thiocyanate met with more hindrance to free diffusion than did heavy water. This is consistent with the concept of restricted diffusion as advanced by Collander(9) and Weech and Michaelis(10) and further developed by Manegold(11) and Pappenheimer (12). The early percentage losses are less and its rate of return is no more rapid than D_2O despite the fact that it is limited to a space which is not only smaller but also is in more intimate contact with the capillaries. Nevertheless, turnover of SCN also is rapid since in most cases total net transcapillary loss was complete and half of this loss had returned to the circulation in less than 10 minutes from the time of injection.

The constant relationship between the mean circulation time of T-1824 and the equilibrium times of SCN and D_2O show that the net transcapillary exchanges of these permeable substances are blood flow dependent. Indeed, it is possible to predict the approximate equilibrium time of these substances when the rate of blood flow is known.

The present method offers several advantages over previously used technics for studying transcapillary exchange. The first is that the studies are conducted in normally functioning untraumatized tissue without use of artificial perfusion fluids. Only tracer doses of the injected materials are used which do not upset the osmotic equilibria of the blood in the capillaries under study. Another desirable feature is that sampling is carried out at intervals of seconds during the early phase. This is important since percentage losses must be determined during the brief period when the concentrations in the blood are higher than those in the tissues. The third advantage is the use of an impermeable tracer to cancel out the effects of dilution by the blood itself. The fourth is limitation of the study to a small vascular area in order to prevent significant recirculation of the tracer substances. As a result following completion of injection the blood entering the forearm will be practically free of labelled materials producing favorable concentration gradients for studying net rates of return of the substances under study. Thus, rate of net loss and rate of net return can be compared in a single experiment. Finally, the method is applicable to any organ or body area where an afferent

and efferent vessel are available for injection and sampling. A modification applicable to animals for preventing significant recirculation in organs with a large blood flow (such as the kidney) will be described in a subsequent communication.

Summary and conclusions. A method is presented for determining under physiological conditions the net bidirectional exchange of permeable tracer substances across the capillary walls of the human forearm. 1. In the early period, maximum net transcapillary loss for thiocyanate was 49 ± 19% and for deuterium oxide 90 ± 4%. 2. The equilibrium time or period of net loss averaged 111 ± 66 seconds for SCN and 130 ± 86 seconds for D_2O. Equilibrium times for both SCN and D_2O were related directly to blood flow. 3. The half-return time for SCN averaged 3 ± 3.0 times the equilibrium time. In the cases in which simultaneous measurements were made the half-return time of D_2O averaged twice that of SCN.

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